Malaysian Journal of Microbiology, Vol 4(1) 2008 pp. 49-52
http://dx.doi.org/10.21161/mjm.02208

SHORT COMMUNICATION
Vegetative compatibility group of *Fusarium* species associated with root and stem rot of Orchid

Latiffah, Z.,* Nur Hayati, M. Z., Baharuddin, S. and Maziah, Z

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
E-mail:lfah@usm.my

ABSTRACT

F. oxysporum, F. proliferatum and *F. solani* isolates were recovered from root and stem rot of orchid. The isolates were placed into vegetative compatibility group (VCG) based on pairing of complementary mutants. From complementation tests, all *F. solani* isolates did not form heterokaryons and was therefore heterokaryon self-incompatible. For both *F.oxysporum* and *F. proliferatum* isolates, four VCG were identified. The results suggest that variation exist among the *F. oxysporum* and *F. proliferatum* isolates from root and stem rot of orchid.

Keywords: VCG, Fusarium, orchid, root, stem rot

INTRODUCTION

Orchid is one of the most widely cultivated ornamentals in Peninsula Malaysia and contributes about 40% of total productions of cut flowers in Malaysia (Lim *et al*., 1998). In a disease survey in nurseries in Penang, root and stem rot disease were commonly observed. Typical symptoms on the root were dark discolouration which indicates rotting of the tissues. Infected stem showed yellowish discolouration with water soaked appearance and very friable. From the disease survey, *Fusarium* species were frequently isolated from the infected orchid which suggested that the fungi are associated with the disease symptoms.

From pathogenicity test by artificial inoculation on root and stem of *Dendrobium* orchid, F*. oxysporum, F. proliferatum* and *F. solani* were able to cause root and stem rot on the orchid. Disease symptoms on the root and stem rot on the *Dendrobium* were similar to those observed in the nurseries (Nur Hayati, 2007). This could be the first recorded root and stem rot of *Dendrobium* caused by *Fusarium* species in Peninsula Malaysia.

There are a number of techniques used to observe genetic variation within fungal pathogens. One of the techniques is VCG which is based on the ability of the mycelium to anastomose to form heterokaryon to determine genetic relatedness (Puhalla, 1985). As the genetic variation of *Fusarium* species causing root and stem rot of orchid in Peninsula Malaysia is poorly understood, the study was undertaken to observe genetic variations based on VCG analysis of *Fusarium* species associated with root and stem rot of orchid.

MATERIALS AND METHODS

Fusarium isolates

Fusarium isolates were isolated from a total of 28 *Dendrobium* orchid showing symptom of root and stem rot, in three nurseries in Penang and Perak. The root and stem samples (about 1.5 – 2.0 cm) were surface sterilized with 1% sodium hypochlorite and rinsed in sterile distilled water. The samples were then plated onto peptone pentachloro nitro benzene agar (PPA) and incubated at 27 ± 1 °C for 7 days or until visible sign of mycelial growth from the samples. The mycelium was then sub-cultured on carnation leaf agar (CLA) for identification. The isolates were identified based on the description of Nelson *et al*. (1983) and Leslie and Summerell (2006).

Vegetative Compatibility Grouping

Besides the isolates recovered from root and stem rot of *Dendrobium*, eight *Fusarium* isolates from USM Culture Collection were included in this study. These cultures were isolated from stem rot of orchid. However, there was no information on the orchid species or variety from which the *Fusarium* isolates was isolated. The cultures were *F. proliferatum* (1325, 1374, 1377, 1378, 1380, 13811), *F. solani* (1257) and *F. oxysporum* (1493) (Table 1). Nitrate non-utilizing (nit) mutants were generated and the phenotypes were characterized as *nit*1, *nit*3 or *nit*M as described by Correll *et al*. (1987). Pairings of all possible combinations or complementation of the nit mutants was made on minimal media (MM) (Puhalla, 1985) and amended with kalium chlorate concentration ranging from 1.5% (w/v) to 5.0% (w/v). The MM plates were incubated at 25 °C. Vegetatively compatible isolates produced abundant aerial mycelium at the interface of two colonies and were placed in the same group.

***Corresponding author**

Table 1: *Fusarium* isolates used in VCG analysis, their host and location

Isolate	Host and Plant part	Location
F. oxysporum		
AP1	Root (*Dendrobium*)	Relau, Penang
AP2	Root (*Dendrobium*)	Relau, Penang
AP3	Root (*Dendrobium*)	Relau, Penang
AP5	Root (*Dendrobium*)	Relau, Penang
AT14	Root (*Dendrobium*)	Taiping, Perak
AT15	Root (*Dendrobium*)	Taiping, Perak
BP1	Stem (*Dendrobium*)	Relau, Penang
BP2	Stem (*Dendrobium*)	Relau, Penang
BP3	Stem (*Dendrobium*)	Relau, Penang
BP4	Stem (*Dendrobium*)	Relau, Penang
BT6	Stem (*Dendrobium*)	Taiping, Perak
BT8	Stem (*Dendrobium*)	Taiping, Perak
BT9	Stem (*Dendrobium*)	Taiping, Perak
1493	Stem (stock culture)	Kuala Lumpur
F. solani		
AP4	Root (*Dendrobium*)	Relau, Penang
AT13	Root (*Dendrobium*)	Taiping, Perak
AT16	Root (*Dendrobium*)	Taiping, Perak
BP5	Stem (*Dendrobium*)	Relau, Penang
BT7	Stem (*Dendrobium*)	Taiping, Perak
BT13	Stem (*Dendrobium*)	Taiping, Perak
1257	Stem (stock culture)	Kuala Lumpur
F. proliferatum		
AP6	Root (*Dendrobium*)	Relau, Penang
AP10	Root (*Dendrobium*)	Relau, Penang
AP11	Root (*Dendrobium*)	Relau, Penang
AP12	Root (*Dendrobium*)	Taiping, Perak
BT10	Stem (*Dendrobium*)	Taiping, Perak
BT11	Stem (*Dendrobium*)	Taiping, Perak
BT12	Stem (*Dendrobium*)	Taiping, Perak
BT14	Stem (*Dendrobium*)	Taiping, Perak
BT15	Stem (*Dendrobium*)	Taiping, Perak
1325	Stem (stock culture)	Kuala Lumpur
1374	Stem (stock culture)	Kuala Lumpur
1377	Stem (stock culture)	Kuala Lumpur
1378	Stem (stock culture)	Kuala Lumpur
1380	Stem (stock culture)	Kuala Lumpur

RESULTS

A total of 155 mutants of *F. oxysporum* were obtained in which 50% were *nit*1, 20% *nit*3 and 30% *nit*M. From complementation test, four VCGs were established that contain 2 – 4 members (Table 2). VCG grouping of *F. oxysporum* was according to the symptoms and locations. Only one *F. oxysporum* isolate, 1493 from the stock culture failed to produce heterokaryon and therefore was classified as heterokaryon self-incompatible. Isolates from *Dendrobium* root were grouped in VCG1 and VCG2. VCG3 and VCG5 contained isolates from *Dendrobium* stem.

For *F. proliferatum*, 135 mutants were obtained which comprised 54% *nit*1, 11% *nit*3 and 35% *nit*M. Only one isolate recovered from stem, BT15 was heterokaryon self-incompatible. Fourteen *F. proliferatum* isolates were grouped in four groups (Table 3) which consisted of 2 – 5 isolates. VCG1 consisted of the stock cultures from Kuala Lumpur and VCG2 consisted of only two cultures, one from the stock culture isolated in Penang (1374) and the other one (BT14) was isolated from stem in Perak. The isolates from stem were grouped in VCG3 and four isolates from roots in VCG4. VCG grouping of *F. proliferatum* isolates did not seems to be correlated with the location except for VCG1 and VCG3.

F. solani isolates did not form thin sector although chlorate concentration was increased to 5%. All the isolates produced thick mycelium which indicated the formation of wild type isolates.

Table 2: VCG of *F. oxysporum* isolates associated with root and stem rot of orchid

Isolate	Plant host	Location
VCG 1		
AP1	*Dendrobium* root	Penang
AP2	*Dendrobium* root	Penang
AP3	*Dendrobium* root	Penang
AP5	*Dendrobium* root	Penang
VCG2		
AT14	*Dendrobium* root	Perak
AT15	*Dendrobium* root	Perak
VCG3		
BP1	*Dendrobium* stem	Penang
BP2	*Dendrobium* stem	Penang
BP3	*Dendrobium* stem	Penang
BP4	*Dendrobium* stem	Penang
VCG4		
BT6	*Dendrobium* stem	Perak
BT8	*Dendrobium* stem	Perak
BT9	*Dendrobium* stem	Perak

Table 3: VCG of *F. proliferatum* isolates associated with root and stem rot of orchid

Isolate	Sources / Plant host		Location
VCG 1			
1325	USM Collection	Culture	Kuala Lumpur
1377	USM Collection	Culture	Kuala Lumpur
1378	USM Collection	Culture	Kuala Lumpur
1380	USM Collection	Culture	Kuala Lumpur
1381	USM Collection	Culture	Kuala Lumpur
VCG2			
BT14	*Dendrobium* stem		Perak
1374	USM Collection	Culture	Penang
VCG3			
BT10	*Dendrobium* stem		Perak
BT11	*Dendrobium* stem		Perak
BT12	*Dendrobium* stem		Perak
VCG4			
AP6	*Dendrobium* root		Penang
AP10	*Dendrobium* root		Penang
AP11	*Dendrobium* root		Penang
AT12	*Dendrobium* root		Perak

DISCUSSION

In the present study, three *Fusarium* species, namely *F. oxysporum, F. solani* and *F. proliferatum* were isolated from root and stem rot of *Dendrobium* orchid. *Fusarium oxysporum* was the most frequent species isolated followed by *F. solani* and *F. proliferatum*. The three *Fusarium* species have been reported to be associated with root rot of *Cymbidium* (Benyon *et al*., 1996), root rot of moth orchid (*Phalaenopsis* sp) (Kim *et al*., 2002) and dry rot of *Cymbidium* (Lee *et al*., 2002).

Genetic variation of the *Fusarium* isolates from root and stem rot of orchid was analysed using VCG. In VCG analysis, both *F. oxysporum* isolates and *F. proliferatum* isolates were grouped into four VCG. Presence of a number of VCG implies that variation exists among the isolates and vegetative compatible isolates demonstrate a high degree of relatedness.

VCG diversity can be calculated by dividing the number of total VCG by the total number of isolates (Smith White *et al*., 2001). In the present study, the overall VCG diversity for *F. oxysporum* was 33% and *F. proliferatum,* 28% which suggest that VCG analysis showed considerable variations among the isolates. In VCG analysis, the variation could be cause by a single base changes with compatible loci which may divide two almost identical isolates into separate groups (Smith-White, 2001).

Isolates in a same VCG often share pathological and physiological traits as well as geographical origins (Swift *et al*., 2002). In this study, this attributes can be seen in *F. oxysporum* isolates in which the VCGs were grouped according to the symptom and locations.

For *F. proliferatum*, the VCGs were grouped according to the symptoms but not the locations. Isolates in VCG2 and VCG4 were from different locations. It is presumed that the isolates in the same VCG to be clones even if the isolates are geographically isolated. This could be that loci and alleles of VCG are selectively neutral with respect to traits such as pathogenicity and vegetative viability (Leslie, 1990).

In the present study, VCG of *F. oxysporum* and *F. proliferatum* were grouped according to the disease symptoms in which it was similar to a study by Venter *et al*. (1992). The study showed that *F. oxysporum* f. sp. *tuberosi* isolates that caused symptoms of stem-rot end, dry rot and wilt on potato could be placed into distinct VCG consistent with the symptoms shown.

Some isolates were unable to form heterokaryon such as *F. proliferatum* (BT15), *F. oxysporum* (1493) and all *F. solani* isolates. These isolates were classified as heterokaryon self-incompatible. According to Leslie (1993), isolates or strains that carry mutations prevent them to form heterokaryons even with themselves, and have been identified in field population of *F. oxysporum, F. moniliforme* and *F. subglutinans.* For *F. solani* isolates, Leslie & Summerell (2006) reported it is difficult to generate *nit* mutants and high proportions of the isolates were heterokaryon self-incompatible. For these isolates

that did not form heterokaryons, molecular markers would provide insight into variations among the isolates.

Additional studies need to be conducted to better characterize the isolates and also to include isolates from more diverse locations and different orchid species. The isolates should also be evaluated by using molecular methods as VCG analysis has its limitations especially some isolates were unable to form mutants on chlorate medium and to form heterokaryon in complementation test.

In conclusion, VCG analysis detected considerable variations in *F. oxysporum* and *F. proliferatum* isolates from root and stem rot of orchid, and *F. solani* isolates were self-incompatible.

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